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Prolonged Mechanical Circumferential Stretch Induces Metabolic Changes in Rat Inferior Vena Cava

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WHAT THIS PAPER ADDS

This study comprehensively characterizes metabolic changes of rat inferior vena cava segments stretched in an *ex vivo* organ bath. Further research into identifying the causes for increased metabolites in conditions representing venous hypertension will allow for identification of the mechanisms of action of each metabolite and possible therapeutic targets that may be beneficial in reducing the progression and recurrence of varicose veins.

Objective/Background: Circumferential stretch on the vein wall has been suggested as a potential etiological factor in the development of varicose veins. However, the influence of vein wall stretch on vein metabolism has not yet been explored. The aim of this study was to investigate the effect of short and prolonged mechanical stretch on vein wall metabolism.

Methods: Circular segments of inferior vena cava from male Sprague–Dawley rats were exposed to normal 0.5-g (nonstretched) or high 2-g (stretched) tension for short (4 h) or prolonged (18 h) duration (five vein segments per group). Contraction response to phenylephrine (10^{-5} M) and KCl (96 mM) was elicited to observe the effect of circumferential stretch on vein function. The polar and organic metabolites in vein tissue were extracted using a bilayer extraction method. Aqueous and organic extracts were analyzed using nuclear magnetic resonance spectroscopy and ultra performance liquid chromatography coupled to mass spectrometry, respectively. Data acquired from both analytical platforms were analyzed using mathematical modeling.

Results: Increased concentrations of valine ($p = .02$) and choline ($p = .03$) metabolites and triglyceride moieties ($p = .03$) were observed in veins stretched for 18 h compared with the nonstretched/18 h group.

Discussion: Increased concentrations of branched chain amino acid valine and cell membrane constituent choline indicate increased muscle breakdown and increased metabolism of membrane phospholipids under stretch in an *ex-vivo* model. Increased intensities of triglyceride moieties in stretched vein segments for 18 h suggest that high pressure may induce an inflammatory response.

Conclusion: This study has shown that prolonged mechanical circumferential stretch (18 h) alters the metabolic profile of rat inferior vena cava.

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INTRODUCTION

Exposure to increased hydrostatic pressure in patients with environmental and genetic risk factors for venous disease is considered fundamentally responsible for disease development and progression.[1–3] Mechanical stretch exerted by venous hypertension may cause damage to the valves directly or may first cause dilatation of vein walls, which then forces the valves apart, causing valvular incompetence.[3]

Mechanical circumferential stretch of cultured vascular smooth muscle cells has been found to regulate their proliferation, apoptosis, and reorganization of extracellular matrix.[4] Zwolak et al. transplanted a rabbit's jugular vein into carotid artery circulation and noted intimal cell proliferation at 1 h, and smooth muscle cell proliferation and vein wall thickening at 2 weeks were observed after transplantation, suggesting that exposure to high pressure (circumferential stretch and shear stress) may be a cause for

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such changes.[5] It has been demonstrated that prolonged stretch increases vein wall tension and upregulates the expression of hypoxia-inducible factor-1α and hypoxia-inducible factor-2α mRNA and proteins in a rat inferior vena cava (IVC) explant model. These factors were associated with increased matrix metalloproteinase expression, increased venous relaxation, and hence dilatation.[6]

Metabolic phenotyping of biological samples can be used to measure the alterations in small molecules or metabolites in response to diet, environmental factors, genetics, and disease.[7–9] Using metabolic profiling (metabonomic) approaches, it has been previously shown that an increased concentration of lactate, creatine, and *myo*-inositol metabolites are identified in human varicose veins samples compared with nonvaricose vein controls,[9] indicating that the technology is appropriate for investigating pathological changes in vein tissue. Here a metabonomic approach is applied to exploring metabolic and vein wall changes in relation to vein wall stretch, to identify key metabolic pathways that may be involved in venous disease and hence potentially identify new therapeutic targets. It is hypothesized that application of increased circumferential stretch (higher tension and hence pressure), will cause alterations in the metabolism of the vein wall compared with non-stretched control (lower tension, normal pressure). To understand the effect of stretch on vein metabolism, an *ex vivo* rat inferior IVC organ culture model was used and vein segments were stretched for 4 and 18 h. Two analytical platforms, nuclear magnetic resonance (NMR) spectroscopy and ultra performance liquid chromatography coupled to mass spectrometry (UPLC-MS) lipid profiling, were used to explore metabolic perturbation in stretched and non-stretched rat IVC segments.

METHODS

Tissue preparation and solutions

Stretching of IVC segments and isometric contractions studies was performed at the Vascular Research Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. All animal research experiments were conducted following the guidelines of the Standing Committee on Animals at Harvard Medical School and followed the Ethical Research Guidelines at the Brigham and Women's Hospital and Harvard Medical School. Krebs solution contained NaCl 120 mM, KCl 5.9 mM, NaH_2PO_4 1.2 mM, dextrose 11.5 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 1.2 mM bubbled with 95% O_2, and 5% CO_2 at pH 7.4. KCl solution (96 mM) was prepared with the same equimolar substitution of NaCl with KCl. The α-adrenergic agonist phenylephrine (PHE) 10^{-5} M was used to stimulate IVC contraction. Tissue culture medium included minimal essential medium supplemented with penicillin streptomycin and amphotericin B (Gibco/Invitrogen, Grand Island, NY, USA). Male 12-week-old Sprague—Dawley rats weighing 250—300 g ($n = 5$, Charles River Laboratories, Wilmington, MA, USA) were euthanized by inhalation of CO_2. Euthanasia was judged by cessation of breathing and heartbeat. The abdominal cavity was opened, and the tissue in the retroperitoneum, including the IVC, aorta, kidneys, and posterior muscles and surrounding fat, was rapidly excised, placed in Kreb's solution, and the IVC carefully dissected and cleaned of all periadventitial tissue and nearby structures under a dissecting microscope. The IVC was portioned into four 3-mm-wide rings in preparation for isometric contraction experiments.

Application of stretch and isometric contraction in rat IVC segments

Each IVC segment was suspended between two tungsten wire hooks; each hook had full contact with the luminal surface of the vein segment providing a circumferential stretch. One hook was fixed to a glass rod at the bottom of the tissue bath and the other was connected to a GRASS Force Displacement Transducer (FT03; Astro-Med Inc., West Warwick, RI, USA). After calibration using a 2-g weight, vein segments were stretched under a predetermined basal tension (0.5 g) at the beginning (nonstretched, 1 h) in a temperature-controlled, water-jacketed tissue bath filled with 50 mL Kreb's solution continuously bubbled with 95% O_2 and 5% CO_2 at 37 °C. The changes in isometric contraction were recorded on a GRASS polygraph (Model 7D; Astro-Med). IVC segments of rats were then subjected to stretch and no stretch by increasing or decreasing the tension to the hook, respectively (Fig. 1).

Contractions were assessed using 96 mM KCl solution, as measured by positive deflection on polygraph, at the end of each experiment for each vein segment. Previous work by Raffetto et al. has demonstrated the response of contraction of rat IVC segments to 96-mM KCl membrane depolarization under the influence of different tensions (0.0625 g to maximum of 3 g).[10] The same protocol was followed and used, 0.5-g tension as the basal tension and 2-g tension as the maximum load.[10] The basal tension of 0.5 g and maximum tension of 2 g are also in concurrence with the venous pressure observed in the superficial venous system of human leg.[6] For example, 0.5-g tension is equivalent to 20.8 mmHg and 2-g tension is equivalent to 83.4 mmHg.[6]

The same protocol for applying stretch and eliciting contractions to rat IVC wall was employed as previously described.[6,10] This included stimulation of IVC segments twice with 96 mM KCl and then PHE 10^{-5} M. Each contraction was followed by a 3-minute wash in Kreb's solution. The IVC segments were then stretched for 4 h. After 4 h of stretch, contractions were stimulated twice with 96 mM KCl followed by PHE 10^{-5} M and tissue was bathed with Kreb's solution between contractions. Two IVC segments, stretched to 0.5-g and 2-g tension, were then stored at −80 °C. The remaining two IVC segments were then stretched with basal tension of 0.5 g and prolonged tension of 2 g for 18 h in culture medium. At 18 h, contractions were assessed by stimulation with 96 mM KCl solution and PHE 10^{-5} M solutions before storing the samples at −80 °C.

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